Declaration in accordance with 37 CFR 1.132

I, the undersigned Sylvia Berman, MSc, a citizen of Israel, residing in Holon, Kiriat Sharet, Israel, hereby declare the following:

1. I have been working at Assaf Harofeh Medical Center, Zerifin, Israel, for the last 35 years. I was initially accepted to work as a lab technician and then, after starting my own lab, have been serving as the Head of Nephrology Research Laboratory for about three decades. At present, this lab has become a part of the Research & Development Unit and as such is always ready to be at service of every basic science project running in our Unit.

2. I was requested by the applicants to undertake measurements of the approximate average dimensions and weight of granules of tattoo ink pigments and to compare my findings with the known approximate average dimensions and weight of typical bacteria found in the relevant literature. All of the experiments reported herein were carried out by me (or under my direct supervision) at Nephrology Research Laboratory which is a part of the Research & Development Unit.

3. In the first stage of the experiment, we received a non-sterile solution of red pigment of origin unknown to us. A small part of the solution was diluted in a sterile PBS buffer (1ml:9ml buffer). Then, a microscope preparation was performed by placing a 5µl drop of the diluted pigment on a glass slide and covering it with a coverglass slip. Visual microscope examination was performed using the Olympus CK X 41 microscope (Japan) at x40 magnification. We could observe the following:

   A. The solution was highly contaminated with bacteria, indicating that the original solution was not prepared at sterile conditions.

B. Each microscope field contained the red pigment crystals of various shapes and dimensions, as well as bacteria and numerous bacterial cell debris.

Since all the latter would interfere with morphometric analyses, we first performed gradient centrifugation of undiluted pigment, thus getting rid of cell debris and of at least part of the bacteria. Subsequently, 5 microliter samples of this solution were placed onto the microscope slides and covered with a coverglass, in order to distribute the drop evenly on the slide surface.

Morphometric evaluations were performed using our computerized morphometry program CMS-2-M which is a part of Advanced Measurement Systems, Ltd (Israel), on Olympus CKX 41 microscope. CMS-2-M system includes digital color CCD camera (1600 x 1200 pixels) and a software package for pathology & immunohistochemistry evaluation. The results were calculated using the CMS-2-M system computerized morphometry program.

In brief,
Mean square area of an average red crystal was calculated based on 15 randomly chosen microscopic fields per slide, by measuring 15 randomly chosen crystals per such field. Subsequently, the result was presented as mean square area ± standard deviation.

Mean square area± standard deviation of an average crystal was 136.59±64.32 square micron.

For reference: the mean square area of an average bacteria is about 0.2 square micron, according to Wikipedia and related sources.

It should be noted that the standard deviation was very high, reflecting the extreme variability of shapes and dimensions of the crystals. In other words, the result should be considered as quite approximate. Still, this is the square area of a single crystal, not an agglomerate of crystals, which would be much larger. Since the average square area of a single bacteria is about 0.2 square micron, the square area of the smallest crystal remains much larger than that of bacteria.

4. In the second stage of the experiment, we were asked to calculate the approximate average weight of the pigment as compared to that of bacteria. In order to do so, we had to possess a pigment solution free of bacteria, i.e. a solution prepared at sterile conditions. We received the red pigment as a powder and prepared a sterile solution in PBS at a concentration of 10μg/ml. The experiment to determine the average weight of a granule of pigment was performed on March 22, 2010. A sterile, freshly prepared, solution of red tattoo pigment looked different from the solution used in the first stage under the microscope: mostly, we saw agglomerates of the pigment instead of single crystals. This, however, was irrelevant to the procedure applied for calculation of the approximate average weight of the pigment agglomerate.

In brief, samples of the prepared solution of 10μg/ml were placed in a standard hemocytometer, the one routinely used in laboratories for cell or cell cluster count. The number of agglomerates of pigment per ml of solution was counted. The average number received from 15 trial measurements was $5x10^7$ (50 million).

The approximate weight of the average agglomerate was calculated as the net mass of ink in one ml divided by the number of agglomerates in one ml:

$$10\mu g/ml : 5x10^7 \text{ agglomerates/ml} = 2x10^{-7}\mu g.$$

For reference: the approximate size of bacteria varies, according to StateMaster Encyclopedia, from 0.5 up to 5micron, its minimal approximate square area being about 0.2 square micron (the routine laboratory precaution for sterile work is to filter fluid through a 0.2 micron filter). According to StateMaster Encyclopedia, the approximate average weight of Escherichia Coli, which are the most common and typical bacteria in the world, is 0.665×10^{-12}µg. It should be emphasized once more that when one deals with such items as bacteria or pigment agglomerates, with very small size and very high coefficient of variability, only approximate average results can be achieved.

5. I hereby declare that all information presented herein is based on my own experience and/or knowledge of the relevant literature, and as such is believed to be true.

6. The name and signature below are my name and signature.

Sylvia Berman, MSc

April 08, 2010

Sylvia Berman MSc
Head of Nephrology
Research Laboratory
R&D
Assaf Harofeh Med. Center